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The Pigment-Dispersing Hormone Family: Chemistry, Structure-Activity Relations, and Distribution

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Abstract. This report summarizes recent work on the pigment-dispersing hormone (PDH) family, a set of related neuropeptides common to arthropods. The primary structures are known for the major form of PDH in several crustacean species (*Pandalus borealis, Uca pugilator, Cancer magister, Penaeus aztecus, Procambarus clarkii*) and for related pigment-dispersing factors from two insects (*Acheta domesticus, Romalea microptera*). In this peptide family, the amino acid chain length (18 residues), termini (N-terminal Asn, C-terminal Ala-NH_2), and at least 50% of the sequence are conserved. Synthetic analogs have been used to analyze the structure-activity relations of PDH, leading to: an evaluation of the role of specific residues; a tentative identification of the message sequence; and the preparation of stable and superpotent analogs including tyrosinated analogs for radioiodination. An enzyme-linked immunosorbant assay (ELISA) has been developed for β-PDH. Antisera raised against α-PDH and β-PDH were used to determine the distribution of PDH. This distribution and other evidence indicate that, besides its role in humoral regulation of the pigmentary system, PDH may serve extra-pigmentary functions. The functions of the PDH-related peptides in insects are unknown.

Introduction

Crustaceans display reversible color changes and eye pigment movements. The color changes result from dispersion or concentration (aggregation) of pigment granules within epithelial chromatophores. The somewhat less conspicuous eye pigment movements, associated with light or dark adaptation, may be restricted to retinular cells (photoreceptor cells), or may also involve extra-retinular ommatidial pigment cells. Whereas pigment movements within retinular cells are induced mainly by a direct action of light, the extra-retinular ommatidial pigment cells as well as epithelial chromatophores are controlled by neurosecretory hormones. The total number of pigmentary-effector hormones present in any given species is unknown, but they are separable into two sets having mutually antagonistic actions. The hormones causing chromatophoral pigment concentration and ommatidial dark adaptation belong to one set, and they are distinct from the hormones eliciting chromatophoral pigment dispersion and ommatidial light adaptation (see reviews: Rao, 1985; Rao and Riehm, 1988a, b). Among hormones in the first set, primary structure is known for only the red pigment-concentrating hormone (RPCH) isolated from eyestalks of the shrimp *Pandalus borealis* (Fernlund and Josefsson, 1972). This crustacean RPCH, an octapeptide (<Glu-Leu-Asn-Phe-Ser-Pro-Gly-Trp-NH_2), is structurally related to insect adipokinetic hormones (AKHs). The functions and structure-activity relations of the RPCH/AKH family peptides are reviewed in this issue by Goldsworthy and Mordue (1989).

Our recent work showed that an additional family of peptides, including the pigment-dispersing hormones (PDHs), is common to crustaceans and insects. This report focuses on the chemistry, structure-function relations, and distribution of PDHs and related peptides.

Structure and Relative Potency of Identified Peptides

When extracts of eyestalks are subjected to cation-exchange chromatography, several zones of PDH activity (3–5, depending on the species) can be detected. The structural basis of this heterogeneity remains unresolved, because in each of the species studied, the amino acid sequence has been deduced for only the major form of PDH. The first structural elucidation in this set of hormones was for an octadecapeptide called light-adapting

Table I

The pigment-dispersing hormone (PDH) family: structure and relative potency

Source	Sequence**	Relative potency*
	1 6 12 18	
Uca pugilator (β-PDH)	N S E L I N S I L G L P K V M N D A amide	1.0
Cancer magister (β-PDH)	N S E L I N S I L G L P K V M N D A amide	1.0
Procambarus clarkii PDH	N S E L I N S I L G L P K V M N E A amide	0.14
Penaeus aztecus PDH	N S E L I N S L L G I P K V M N D A amide	1.0
Acheta domesticus PDF	N S E I I N S L L G L P K V L N D A amide	0.5
Romalea microptera PDF	N S E I I N S L L G L P K L L N D A amide	0.5
Pandalus borealis (α-PDH)	N S G M I N S I L G I P R V M T E A amide	0.05

* Based on assays for pigment dispersion in melanophores of *Uca pugilator*; β-PDH = 1.0.

** Notation is the approved one-letter abbreviations for the amino acids. Underlined residues are those different from β-PDH.

distal retinal pigment hormone (DRPH) from eyestalks of the shrimp *Pandalus borealis* (Fernlund, 1976). This peptide elicits not only ommatidial light adaptation, but also chromatophoral pigment dispersion (Kleinholz, 1975), and is referred to as α-PDH in the recent literature.

An octadecapeptide differing from α-PDH at six positions, and designated β-PDH, has been identified as the major form of dispersing hormone in extracts of eyestalks from the crabs *Uca pugilator* (Rao *et al.*, 1985) and *Cancer magister* (Kleinholz *et al.*, 1986). Recent work in our laboratory indicated that the major forms of PDH in eyestalks of the brown shrimp *Penaeus aztecus* (Phillips *et al.*, 1988) and the crayfish *Procambarus clarkii* (McCallum *et al.*, 1988) have more sequence similarity to β-PDH than to α-PDH (Table I). Because of the substitution of Glu^3 for Gly^3, the PDHs of *Penaeus* and *Procambarus*, as well as β-PDH, are more acidic and elute faster than α-PDH in cation-exchange chromatography.

When the cation-exchange chromatography profiles were compared, α-PDH could not be detected in eyestalk extracts from *Uca*, *Cancer*, *Penaeus*, and *Procambarus*. Yet in *Pandalus*, α-PDH is the identified major form. Two faster eluting PDH zones are evident in cation-exchange chromatography of *Pandalus* eyestalk extracts, but their structural similarity to β-PDH has not been established.

Since insect head extracts are known to cause melanophore pigment dispersion in *Uca*, we have determined the primary structures of the active peptides (pigment-dispersing factors; PDFs) from two insect species: the lubber grasshopper *Romalea microptera* (Rao *et al.*, 1987), and the domestic cricket *Acheta domesticus* (Rao and Riehm, 1988b). In the latter study, we were able to identify 17 of the 18 residues, and the unidentified residue was presumed to be Arg^{13} or Lys^{13}. More recently, by comparison with synthetic peptides, we concluded that Lys is present as residue 13 in *Acheta* PDF.

The sequence similarity between insect PDFs and crustacean PDHs (Table I) shows that both groups are constituents of an authentic peptide family common to arthropods. This peptide family displays conservation of amino acid chain length (18 residues), conservation of termini (amino-terminal Asn and carboxyl-terminal Ala-amide), and at least 50% sequence similarity. The insect PDFs, like the PDHs of *Penaeus* and *Procambarus*, are more closely related to β-PDH than to α-PDH.

Since the function of PDFs in insects is unknown, these peptides, as well as crustacean PDHs, have been tested for their relative potencies by assays for melanophore pigment dispersion in *Uca*. In these assays the ratio of the highest to lowest relative potency among the PDH family peptides (*i.e.*, β-PDH/α-PDH) is 20 (Table I). *Penaeus* PDH and β-PDH are the most active and are equipotent; thus the two substitutions in the *Penaeus* peptide (Leu^8 for Ile^8, and Ile^{11} for Leu^{11}) are not critical for the potency of β-PDH. The insect PDFs, which differ from β-PDH at three or four positions, are about 50% as potent as β-PDH. Among the individual substitutions noted in the PDH family, the replacement of Asp^{17} by Glu^{17} (*e.g.*, in *Procambarus* PDH) caused a marked decline (7-fold) in potency. The least potent peptide, α-PDH, contains not only Glu^{17}, but also substitutions at five other positions (Table I).

Structure-Activity Relations of Synthetic PDH Analogs

To evaluate the contributions of specific residues to the potency differences between α-PDH and β-PDH, we synthesized several structural intermediates. Although, as noted above, replacement of Asp^{17} by Glu^{17} reduces the potency of β-PDH, replacement of Glu^{17} by Asp^{17} did not increase the potency of α-PDH. Similarly, β-PDH-

related substitutions at three other positions (Leu^{11}, Lys^{13}, or Asn^{16}) did not notably alter the potency of α-PDH. Substitutions at positions 3 and 4 (Glu^3, Leu^4) were each able to increase the potency of α-PDH (2.4 and 3.3-fold, respectively), but they did not account for the net increased potency of β-PDH. The lower potency of α-PDH appears to be due to an interactive effect of multiple substitutions.

As reviewed earlier (Rao and Riehm, 1988b), we have synthesized and assayed a number of additional analogs to evaluate the role of specific substitutions. These studies indicate that the increased potency resulting from the substitution of Glu^3 for Gly^3 may be due to a more stable ligand-receptor interaction through the formation of an ionic bond. The increased potency resulting from Leu^4 substitution for Met^4 is attributable to protection from oxidation. In α-PDH, replacement of either Met^4 or Met^{15} by norleucine (Nle) caused a three-fold increase in potency, whereas replacement of both residues led to a six-fold increase in potency. In β-PDH, which contains a single methionine residue (Met^{15}), the substitution of Nle^{15} imparted hyperpotency (16-fold) and full protection from oxidation. The latter feature proved useful in preparing tyrosinated analogs of PDH, such as the N-terminally extended [N-Tyr-Nle^{15}]-β-PDH, which could be iodinated without loss of biological activity (Rao and Riehm, 1988b).

The PDH family members share common termini, which appear to be crucial for the full potency of these peptides. Tests with truncated analogs of α-PDH showed that deamidation or deletion of C-terminal Ala, or deletion of N-terminal Asn, results in a substantial loss of potency. Further truncation produced very weak agonists, with the analog 1-9-NH_2 being the smallest carboxyl-terminal deletion peptide to display activity (0.001% potency, relative to α-PDH) and the analog 6-18-NH_2 being the smallest amino-terminal deletion peptide with activity (0.03% potency). Since these two truncated peptides share the sequence of residues 6 to 9, and because α-PDH and β-PDH share the hexapeptide sequence of residues 5–10, this region seemed to be the possible message sequence of PDH (Rao and Riehm, 1988b).

Recent work in our laboratory by Zahnow (1987) showed that peptides 5-9-NH_2, 5-10-NH_2, 6-9-NH_2, and 6-10-NH_2 were each able to cause dispersion of melanophore pigments in *Uca*, but were ineffective on leucophores. Thus, although residues 6–9 may constitute the message sequence required for melanophore activation, one or more other components of the octadecapeptide sequence may be needed for the multiple actions of PDH. More detailed studies are needed to elucidate the critical structural and sequence requirements for activation of different pigmentary effector cell types.

Immunocytochemistry

Antisera raised against α-PDH were used to detect immunoreactive somata in the brain and various optic ganglia of the crayfish *Orconectes immunis* (Schueler *et al.*, 1986). The application of β-PDH antiserum revealed immunopositive perikarya in the eyestalk ganglia and in various central ganglia of *Orconectes limosus* and *Carcinus maenas* (Mangerich *et al.*, 1987; Mangerich and Keller, 1988). These studies indicate that PDH is not only associated with neurosecretory cells terminating in the sinus gland, but is also found in apparently non-secretory neurons and in fibers not associated with neurohemal release sites. Therefore, in addition to its well known role as a blood-borne pigmentary-effector hormone, PDH may also serve as a neuromodulator or transmitter.

In eyestalk ganglia of *Orconectes limosus, Carcinus maenas* (Mangerich *et al.*, 1987), and *Penaeus aztecus* (Phillips *et al.*, 1987), some of the PDH-positive soma and nerve tracts were also reactive to a FMRFamide antiserum. In the central ganglia examined in *Orconectes*, PDH and FMRFamide immunoreactivities were not co-localized (Mangerich and Keller, 1988).

Comparable studies in the lubber grasshopper *Romalea microptera* (Zahnow *et al.*, 1987) showed that PDH-positive cells are restricted to the optic lobes, and that some of these cells are also reactive to FMRFamide antiserum. Other cells containing immunoreactive FMRFamide were widely distributed, occurring in the brain, as well as in the optic lobes of *Romalea*.

In the tobacco hawkmoth *Manduca sexta*, PDH immunoreactivity and gastrin/CCK-like immunoreactivity were co-localized in many somata that were distributed widely in the optic lobes, brain, and subesophageal ganglion (Homberg *et al.*, 1987). The physiological significance of the co-localization of immunoreactivities, and the differential distribution of PDH in the two insect species, merits further exploration.

Immunoassays

With an antiserum raised against synthetic β-PDH (Dircksen *et al.*, 1987), we developed an enzyme-linked immunosorbant assay (ELISA) and used it to evaluate antibody specificity (Bonomelli *et al.*, 1988). In competitive tests, the antiserum (diluted 1:100,000) recognized β-PDH with an IC_{50} of 160 fmol/well, but had little affinity for α-PDH (<0.001% relative to β-PDH). The antiserum showed considerable affinity for insect PDFs (13–21%) and *Penaeus* PDH (75%), which are very similar in sequence to β-PDH. The markedly lower affinity (0.4%) noted with *Procambarus* PDH was reminiscent of the low potency as a pigment disperser and could be due to its single substitution—Glu^{17} for Asp^{17}. When α-PDH

analogs containing β-PDH-related substitutions were tested, the analogs containing Asn^{16} or Asp^{17} reacted better than those with Glu^{3}, Leu^{4}, Leu^{11}, or Lys^{13}. The antiserum failed to recognize a C-terminally truncated analog of β-PDH (1-13-NH_2). These findings suggest that the antiserum needs several of the residues closer to the C-terminus in β-PDH for recognition. The specificity of the antiserum raised against α-PDH (Schueler *et al.*, 1986) was not reported.

Extra-Pigmentary Functions

The RPCH/AKH family members show considerable sequence homology, but serve distinct functions in different arthropods: chromatophoral pigment concentration in Crustacea; hyperglycemia, hypertrehalosemia, hyperlipemia, and cardioacceleration in insects (see Goldsworthy and Mordue, 1989). Since insects lack a chromatophoral system, the PDH functions (yet undetermined) are most likely to be extra-pigmentary in these animals. Immunocytochemical distribution points to a role as neuromodulator or neurotransmitter for PDH.

Recent work indicates that RPCH and PDH may also serve extra-pigmentary functions in Crustacea. RPCH has strong excitatory effects on the stomatogastric ganglion (Nusbaum and Marder, 1988). RPCH and β-PDH have stimulatory and inhibitory effects, respectively, on the secretion of methyl farnesoate by crustacean mandibular organs (Laufer *et al.*, 1987), and thus seem to have a role in the regulation of reproduction.

Perspectives

Now that the major PDH-like peptides in several species have been sequenced, and their structure-activity relations, immunoreactivity, and tissue distributions have been determined, a new arthropodan peptide family has emerged. However, because this is a very new family, most aspects of its distribution, functions, and evolution remain to be explored.

The first major problem is to define the limits of the PDH family and the variability within it. To those ends, the amino acid sequences of the unknown multiple forms of PDH in selected species must be determined, and the genetic basis of those sequences will also have to be established. Moreover, the PDH-related peptides—and the genes encoding them—should be sought in a wider selection of arthropods and crustaceans, as well as in other phyletic groups.

Second, although the pigmentary effects of the PDH family will continue to be an object of study, the extra-pigmentary effects will have to be investigated. Sites of action can be located by immunocytochemistry, and functions may be identified by correlating immunoreactive hormone titers with various physiological states. Finally, PDH receptors and receptor mechanisms must be characterized.

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